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Review

Stem Cells for Spinal Cord Repair

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Spinal cord injury typically results in permanent disability. Many studies have indicated that transplantation of several different types of stem cells promotes functional recovery in animal models of spinal cord injury. A conceptually different approach to utilize stem cells for regenerative therapies may be recruitment of endogenous neural stem cells resident in the adult spinal cord. We discuss the possibilities, risks, and mechanisms for stem cells in spinal cord repair.

The spinal cord is the main relay for signals between the brain and the body. Spinal cord injury completely or partially deprives the individual of mobility and sensory input as well as autonomic nervous system control below the level of the lesion. The majority of spinal cord injuries affect the cervical segments, leaving the patient para- or tetraplegic depending on the exact level of the injury (Figure 1). The middle cervical segments control breathing (the motoneurons that subserve the phrenic nerves are located in segments C3–C5), and complete lesions above this level are lethal unless assisted breathing is provided from immediately after the injury. Spinal cord injury is most commonly caused by high-energy trauma, for example from sports or traffic accidents, and the majority of patients are 10–40 years old at the time of the injury. The annual incidence is 15–40 per million, and since the long-term survival is good in developed countries, there are a large number of people who are chronically disabled by spinal cord injury (Sekhon and Fehlings, 2001). There is currently no curative therapy, and the care in the acute phase is limited to high-dose corticosteroid treatment to reduce inflammation and surgical stabilization and decompression to reduce further damage. In the subacute to chronic phase, treatment focuses on symptomatic relief (against, for example, pain and opportunistic infections) and physiotherapy (Baptiste and Fehlings, 2007). Spinal cord injury results in enormous personal suffering and, due to its chronic disabling nature, substantial cost to society.

A combination of factors is responsible for the lack of neural regeneration and minimal functional recovery generally observed after spinal cord injury. The injury severs axons, and the distal segment of the axon (which is isolated from the neuronal cell body) degenerates. The proximal axon segment typically survives but fails to regrow and reinnervate its targets. The lack of axonal regeneration is not primarily due to an inherent lack of axonal growth potential, but rather the presence of axonal growth inhibitors in the adult central nervous system. Myelin-associated proteins and the glial scar, which forms at the injury site and in denervated axonal tracts, inhibit axonal growth (Busch and Silver, 2007; Fawcett, 2006; Kaneko et al., 2006; Schwab, 2004).

The role of the glial scar is complex and has been discussed for more than a century. Strong support for the glial scar as a major negative factor after spinal cord injury comes from the identification of chondroitin sulfate proteoglycans as axonal growth-inhibiting molecules expressed by reactive astrocytes (Busch and Silver, 2007). Moreover, studies of mutant mice lacking the genes encoding the intermediate filament proteins glial fibrillary acidic protein (GFAP) and Vimentin, highly expressed by reactive astrocytes, demonstrated impaired scar formation accompanied by enhanced axonal sprouting and improved functional recovery (Menet et al., 2003; Pekny et al., 1999). However, other studies have shown a beneficial role of the glial scar during the acute phase (1–2 weeks) after spinal cord injury. Elimination of reactive astrocytes or preventing their migration and scar formation after injury resulted in a failure of blood-brain barrier repair accompanied by massive inflammatory cell infiltration and increased loss of neurons and oligodendrocytes with a worse functional outcome (Faulkner et al., 2004; Okada et al., 2006). Moreover, transgenic mice showing enhanced astrocyte migration and premature glial scar formation facilitated recovery (Okada et al., 2006). Thus, an acute astrocytic response appears important to limit and restrain the inflammatory response, but this may be at the expense of reduced axonal regrowth. The glial scar reduces the possibility of grafted cells to migrate and integrate. The majority of studies have therefore investigated the effect of transplanting cells 1–2 weeks after injury (Ogawa et al., 2002). It is more difficult to envisage a cell-based therapy in the chronic phase (Okano, 2002).

In addition to axon transection, spinal cord injury causes death of multiple cell types at or close to the site of injury. Much of the cell death is due to secondary mechanisms, often triggered by ischemia, such as lipid peroxidation, edema, and an increase in free radicals and excitotoxic levels of transmitters (Sekhon and Fehlings, 2001). The loss of neurons, which are not regenerated in the adult spinal cord, results in impaired function of the affected segment. However, probably more deleterious than the neuronal death is the loss of oligodendrocytes and deficient expression of myelin-associated genes. Spinal cord injuries are most commonly incomplete in man, leaving spared tissue connecting the spinal cord above and below the lesion, but the function of remaining axons is often compromised due to demyelination. Without insulating sheaths of myelin, spared axons close to but not directly affected by the injury become less efficient in their ability to conduct electrical impulses (McDonald and Belegu, 2006). Voltage-gated ion channels are arranged in the axonal membrane in relation to the myelin sheath, with sodium channels clustered in the short stretches of axon segment between two adjacent myelin

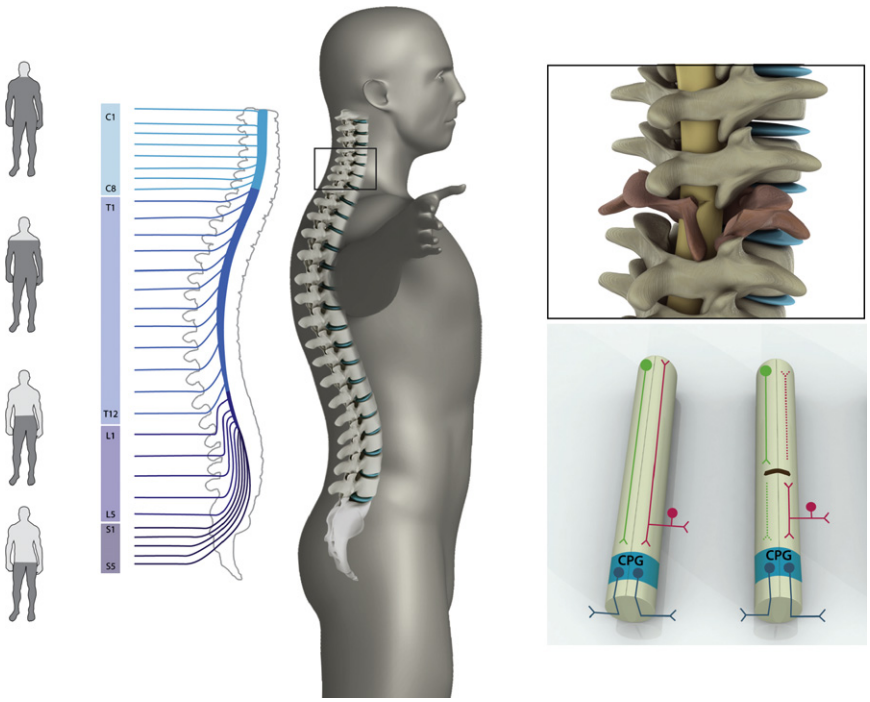

Figure 1. Spinal Cord Injury Results in Disruption of Motor Control and Sensory Input below the Level of the Lesion

Spinal cord injury is most often caused by trauma, which results in dislocation of vertebrae and damage to the spinal cord. Motor control and sensory input is lost below the level of the lesion (shaded area in figures to the left) due to the severance of long ascending sensory (red) and descending motor (green) axons. The local circuitry, including central pattern generators (CPG) responsible for coordinated locomotor function, remains largely intact in uninjured segments.

sheaths (nodes of Ranvier) and potassium channels in the paranodal region. Demyelination results in rearrangement of ion channels, which further impairs propagation of action potentials (Nashmi and Fehlings, 2001). Moreover, chronically demyelinated axons are vulnerable to degeneration. This becomes apparent in multiple sclerosis, where axonal and neuronal loss is thought to be caused by the demyelination (Grigoriadis et al., 2004).

The effects of axon transection, neuronal death, and demyelination on overall signal transmission are compounded by other tissue reactions to the injury, including inflammatory and immune responses, cyst formation, and vascular changes (Sekhon and Fehlings, 2001). Given this complex nature of spinal cord injury, many conceptually different ways to facilitate recovery have been investigated. It is unlikely that any one strategy alone will lead to very dramatic functional improvement. However, even seemingly small improvements in function may have a large impact on a patient's life. For example, the ability to grip with the hands may make the difference of being able to live an independent life or not. Regaining arm and hand function has the highest priority for quadriplegics, and regaining sexual function has the highest priority for paraplegic patients (Anderson, 2004).

Here, we will focus on the advantages, opportunities, and challenges presented by stem cell-based therapeutic strategies for spinal cord injuries, and the reader is referred to recent reviews covering other therapeutic strategies (Fawcett, 2006; Rossignol et al., 2007; Schwab, 2004). There are two conceptually different ways to employ stem cells for spinal cord repair. First, one may transplant stem cells, or cells derived from stem cells, to the injured spinal cord. Second, endogenous neural stem cells resident in the adult spinal cord could potentially be recruited or modulated to promote recovery. We will discuss these two possible avenues for stem cells in spinal cord repair. A much larger number of studies have addressed the possibility to improve recovery after spinal cord injury by stem cell transplantation, rather than recruitment of endogenous cells, and this topic has been the subject of several recent reviews (Enzmann et al., 2006; Okano et al., 2003; Parr et al., 2007; Sharp and Keirstead, 2007). We therefore limit the description of stem cell transplantation strategies to summarize the efficacy and mechanistic insights.

Transplantation of Stem Cells and Stem Cell-Derived Cells to the Injured Spinal Cord

Attempts to improve recovery after spinal cord injury by transplanting cells or tissue has a long history, and several approaches have been taken to clinical trials (reviewed in Tator, 2006). Transplantation of, for example, autologous or fetal tissue is, however, often impractical for large-scale clinical use. Stem cells offer an attractive alternative, not least because of their potential unlimited supply, which may allow the development of more easily applied therapies.

A large number of studies have evaluated the effect of transplanting a variety of stem cells or stem cell-derived cells in spinal cord injury models, mainly in rodents, and remarkably, many studies using different strategies have indicated beneficial effects. There are difficulties in directly comparing studies because of the varying degree of characterization of the transplanted cells, different injury models, and transplantation at different time points after the injury. The evaluation of the effect has also often been done in different ways or to different degrees.

In spite of many studies indicating a beneficial role of stem cell transplantation in spinal cord injury, there is still limited mechanistic insight. The simplest way to explain how transplanted cells may promote recovery is by replacement of lost cells. This is probably an important factor in several strategies, mainly where lost oligodendrocytes are replaced and remyelination by graft-derived cells have been demonstrated (Sharp and Keirstead, 2007) (Figure 2). However, in other situations, functional benefits have been reported without any obvious replacement of neural cells. This is most striking in the case of nonneural cells, such

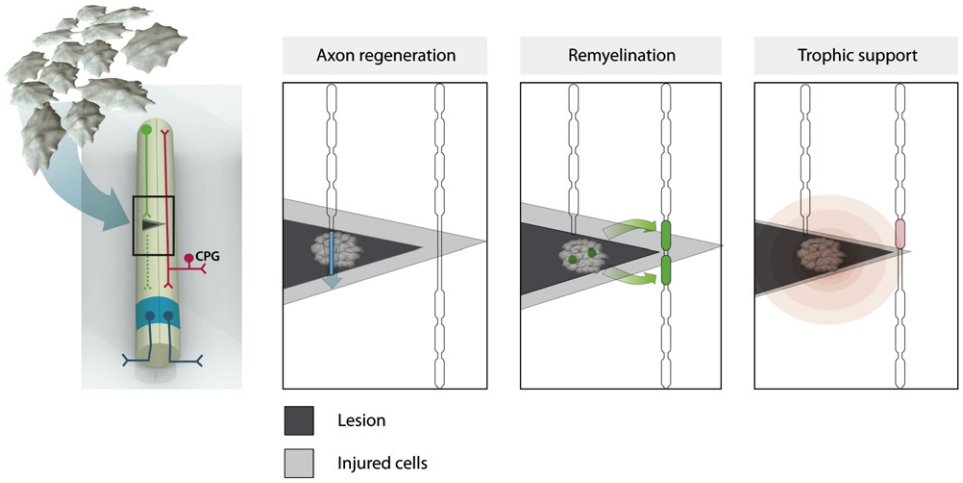

Figure 2. Mechanisms by which Transplanted Stem Cell-Derived Cells May Facilitate Regeneration
It is often difficult to establish the mechanism by which transplanted cells may facilitate functional recovery. Likely mechanisms include creating a permissive substrate for axonal growth, providing cells that remyelinate spared but demyelinated axons, and supplying trophic support reducing the damage and rescuing, for example, neurons and oligodendrocytes. Transplanted cells may in addition (not depicted) enhance axonal plasticity and replace lost neurons to reconstruct local circuitry.

as mesenchymal cells or macrophages, where no neurons or glial cells were generated. The mechanism here appears to be indirect, such as providing trophic support, modulating the inflammatory response, or providing a substrate for axonal growth (Parr et al., 2007) (Figure 2).

The pluripotency and possibility of virtually unlimited expansion of embryonic stem cells (ESCs) make them attractive for the derivation of cells for transplantation to the injured spinal cord. Oligodendrocyte progenitor cells can be efficiently derived from murine and human ESCs (Brüstle et al., 1999; Nistor et al., 2005) and give rise to mature myelinating oligodendrocytes when transplanted to the central nervous system (Brüstle et al., 1999; Keirstead et al., 2005). McDonald, Choi, and colleagues first evaluated the potential of neuralized mouse ESCs for promoting recovery after spinal cord injury and demonstrated in situ oligodendrocyte differentiation and functional improvement (McDonald et al., 1999). More recently, human ESC-derived oligodendrocyte progenitor cells transplanted to the rat were shown to similarly result in oligodendrocyte differentiation, remyelination, and locomotor improvement following acute (7 days after injury), but not chronic (10 months) transplantation (Keirstead et al., 2005).

ESCs are well suited to produce neural progeny, but the caveats with using allogeneic cells has fueled interest in exploring the neuroregenerative potential of adult somatic stem cells. There are many studies that describe transplantation of cells, with varying degree of characterization when it comes to stem cell features, from, for example, bone marrow, umbilical cord, blood, and skin to the injured spinal cord (Parr et al., 2007). The initial rationale for transplanting, for example, bone marrow-derived cells to the injured spinal cord was based on several reports indicating that both hematopoietic stem cells and mesenchymal cells had the potential to generate neurons and glial cells. However, a large number of subsequent studies failed to reproduce the initial results or provided alternative explanations as to the expression of neural markers by bone marrow-derived cells (Meletis and Frisén, 2003). Nevertheless, although bone marrow-derived cells do not appear to generate appreciable numbers of neural cells, there are many studies reporting functional improvement after transplantation of mesenchymal cells to the injured spinal cord (Parr et al., 2007). Rather than being a replacement therapy, transplanted mesenchymal cells appear to affect spinal cord repair indirectly, and suggested mechanisms include production of growth factors, cytokines, and neurotrophic factors; promotion of proliferation of endogenous progenitor cells; and the generation of favorable substrate for axonal growth or effects on the vasculature (Parr et al., 2007).

Many studies have investigated the effects of grafting in vitro propagated neural stem cells or committed neuronal or glial progenitors to the injured spinal cord. Most of the studies performed in rodents that have investigated recovery demonstrate a beneficial effect of the grafted cells on locomotor function. The transplanted neural stem cells typically give rise mainly to astrocytes and more limited numbers of neurons or oligodendrocytes (Enzmann et al., 2006; Hofstetter et al., 2005; Karimi-Abdolrezaee et al., 2006; Ogawa et al., 2002; Pfeifer et al., 2006). Astrocytes produce several neurotrophic factors, and one likely mechanism by which transplanted neural stem cells may promote recovery is by sustaining the survival of host cells and potentially by supporting some local axonal sprouting (Hofstetter et al., 2005). Transplanted neural stem cells also give rise to oligodendrocytes after transplantation, although typically to a lesser degree (Enzmann et al., 2006; Hofstetter et al., 2005; Karimi-Abdolrezaee et al., 2006; Ogawa et al., 2002; Pfeifer et al., 2006). Remyelination and the associated reorganization of axonal ion channels likely contribute to the recovery of some function after neural stem cell transplantation (Eftekharpour et al., 2007). Promoting the generation of oligodendrocyte at the expense of astrocytes by transplanted neural stem cells further facilitates sensory and locomotor recovery (Hofstetter et al., 2005).

Recruitment of Endogenous Spinal Cord Neural Stem Cells for Repair

The presence of neural stem cells in the adult central nervous system raises the possibility that modulation of endogenous stem cells may offer an alternative therapeutic strategy to cell transplantation. Transplantation of in vitro propagated neural stem cells derived from the adult spinal cord can promote functional recovery in rodent injury models (Hofstetter et al., 2005), demonstrating that endogenous adult spinal cord stem cells can facilitate functional recovery but normally fail to do so efficiently. A tantalizing prospect is to in situ, without any cell culture or grafting, reproduce the effect induced by the expansion and transplantation of the endogenous neural stem cells to promote recovery. This would offer a noninvasive autologous therapy that could circumvent many of the limitations and risks with transplantation strategies. However, it is difficult to predict today whether this is a realistic scenario.

The evidence for the existence of multipotent neural stem cells in the adult spinal cord is largely based on in vitro studies. Cells with sustained self-renewal capacity and the potential to differentiate into neurons, astrocytes, and oligodendrocytes can be propagated in vitro (Johansson et al., 1999; Shihabuddin et al., 1997; Weiss et al., 1996). Such cells with in vitro neural stem cell properties are present throughout the rostrocaudal axis of the adult spinal cord (Shihabuddin et al., 1997; Weiss et al., 1996). Neural stem cells propagated from the adult spinal cord differ to some degree from the more well-studied forebrain neural stem cells, where neurogenesis is maintained throughout the organism life. Whereas forebrain neurospheres only require EGF (or related growth factors) as mitogen, adult spinal cord stem cell isolation requires FGF-2 (Shihabuddin et al., 1997; Weiss et al., 1996). Neural stem cells expanded in vitro also differ in their expression of positional markers depending on the level of the neuraxis from which they derive, and this identity is maintained to at least some degree through passaging (Hitoshi et al., 2002).

Resident cells are activated and produce progeny in response to spinal cord injury, but the lack of functional recovery after a lesion all too clearly demonstrates that this is not sufficient to promote regeneration. In fact, there is no evidence today that recruitment of endogenous cells is beneficial at all, and it is possible that it rather affects the outcome negatively, for example, by contributing to scar formation. To be able to develop rational strategies to modulate endogenous neural stem cells in the adult spinal cord, it is necessary to identify adult spinal cord stem and progenitor cells and gain detailed knowledge of their function and response to injury as well as their molecular regulation.

Identity of Adult Spinal Cord Neural Stem Cells

The identity of the cells in the adult spinal cord that display neural stem cell properties in vitro is becoming increasingly clear. Microdissection or injection of a fluorescent label indicated that all, or close to all, neurosphere-initiating cells reside close to the central canal, the narrow extension of the ventricular system spanning the length of the spinal cord (Johansson et al., 1999; Martens et al., 2002). Other studies, in contrast, reported that similar numbers of clones could be propagated from both the medial and lateral parts of the spinal cord (Yamamoto et al., 2001a; Yoo and Wrathall, 2007). However, whereas the neurospheres from the central canal region are multipotent and can be expanded for many passages (Johansson et al., 1999; Martens et al., 2002; Meletis et al., 2008), the clones analyzed from the medial and lateral aspects of the spinal cord could only be expanded for two passages, and only a minority were multipotent with the capacity to generate neurons (Yamamoto et al., 2001a; Yoo and Wrathall, 2007). This suggests the existence of at least two different cell populations: one multipotent population with extended self-renewal capacity residing close to the central canal, and another population with more limited self-renewal capacity, mainly restricted to glial lineages, present in the parenchyma.

Several lines of evidence suggest that the parenchymal progenitors can be found within a population of cells expressing one or several of the partly overlapping markers NG2, Olig2, and Nkx2.2, and that they constitute the majority of proliferating cells in the uninjured adult spinal cord (Ohori et al., 2006) (Figure 3). These cells appear somewhat heterogeneous, with some being committed to the oligodendrocyte lineage and others possessing a broader glial or even neuronal differentiation potential (Horky et al., 2006; Ohori et al., 2006).

Most of the studies to date addressing the identity of adult spinal cord neural stem cells have used indirect methods to label cells. An important limitation in many studies is the lack of cell type specificity with the detection methods employed to study endogenous stem/progenitor cells (Breunig et al., 2007). Studies that have used BrdU incorporation to label dividing cells have helped to identify cells that proliferate under normal circumstances and in response to injury but cannot provide information on lineage relationships (Horky et al., 2006; Horner et al., 2000; Yang et al., 2006). Retroviral infection permanently labels progeny of proliferating cells without cell-type specificity. It is not possible to inject virus without inducing injury at the same time, which is why it is mostly useful for studies in the injured situation (Horky et al., 2006; Ohori et al., 2006). Finally, labeling cells with fluorescent labels or using transgenic animals expressing reporter genes such as *LacZ* or *GFP* under the control of specific promoters does not allow long-term tracking of the progeny of targeted cells, as the labels are diluted or reporter genes are not expressed by the progeny (Frisén et al., 1995; Johansson et al., 1999; Mothe and Tator, 2005). Important knowledge will be gained by using the rapidly expanding number of cell type-specific inducible labeling systems, where specific promoters are used to drive, for example, the expression of inducible Cre recombinase. We have recently generated mice driving the expression of tamoxifen-inducible Cre (CreER) under the control of *Nestin* or *FoxJ1* regulatory sequences (Carlén et al., 2006; Meletis et al., 2008). In both these transgenic lines, CreER expression is confined to ependymal cells in the adult spinal cord. Derivation of neurospheres from such mice, in which ependymal cells were genetically labeled, demonstrated that close to all neural stem cell activity capacity resides within that cell population under standard neurosphere conditions (Meletis et al., 2008). There are also mice expressing inducible Cre under the control of the *Olig2* promoter (Takebayashi et al., 2002), which should aid in the elucidation of the role of candidate parenchymal progenitor cells.

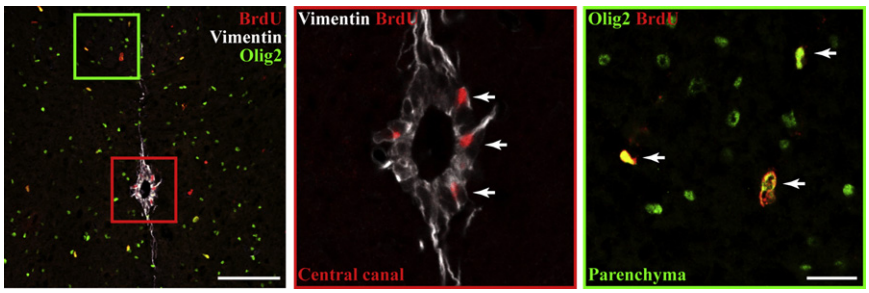

Figure 3. Cell Proliferation in the Adult Spinal Cord
There is little cell division in the uninjured adult mouse spinal cord, but both vimentin+ ependymal cell and Olig2+ parenchymal progenitor cell proliferation is apparent (McTigue et al., 2001; Ohori et al., 2006; Yamamoto et al., 2001b; Meletis et al., 2008) after administering BrdU for 4 weeks in the drinking water. Arrows point to double-positive cells. Scale bars, 100 μm (left panel) and 20 μm (right panel).

Response of Endogenous Neural Stem/Progenitor Cells to Injury

Spinal cord injury results in rapid cell loss at the lesion, with typically about 50% of astrocytes and oligodendrocytes, and an even larger proportion of neurons, being lost already within the first day in rodent injury models (Grossman et al., 2001; Lytle and Wrathall, 2007). The number of oligodendrocytes increases with time, and many axons become remyelinated and astrocyte numbers increase as the glial scar forms (Figure 4). Pre-existing oligodendrocytes do not divide, and proliferation of astrocytes appears limited, indicating that oligodendrocytes and astrocytes, at least in part, are regenerated from stem or progenitor cells after a spinal cord injury (Blakemore and Patterson, 1978; Horky et al., 2006; Keirstead and Blakemore, 1997; Yang et al., 2006).

Cell proliferation is rather limited in the intact spinal cord and is thought to serve a low-grade turnover of glial cells. After injury, a peak of cell division is observed 1–3 days following injury, and cells in at least three distinct locations proliferate at this time: in the ependymal region surrounding the central canal, the parenchyma, and the periphery (Horky et al., 2006). An increased number of neurospheres can be isolated from the injured spinal cord, suggesting that neural stem/progenitor cells proliferate in response to injury (Xu et al., 2006).

Ependymal cells divide rarely in the uninjured spinal cord (Figure 3), but a lesion induces a massive increase in their proliferation within 24 hr (Horky et al., 2006; Johansson et al., 1999; Kojima and Tator, 2002). The majority of proliferating ependymal cells have a cleavage plane parallel to the ependymal surface after a spinal cord injury, suggesting that one daughter cell may remain in the ependymal layer as one daughter cell migrates away (Johansson et al., 1999). Asymmetric cell division can result in differential distribution of fate determinants in the two daughter cells, allowing one to maintain stem cell features and the other to differentiate (Doe, 2008). Ependymal cells that divide in response to spinal cord injury appear to segregate Notch1, a well-characterized receptor associated with neural stem cell features (Louvi and Artavanis-Tsakonas, 2006), asymmetrically to the daughter cell that remains in the ependymal layer (Johansson et al., 1999). Tracing of ependymal cell progeny have demonstrated migration to the lesion site, where the majority of cells differentiate to astrocytes and contribute to scar formation (Frisén et al., 1995; Johansson et al., 1999; Meletis et al., 2008; Mothe and Tator, 2005). Ependyma-derived astrocytes constitute approximately 20% of the cells at the lesion site 2 weeks after the injury and appear to remain there permanently. Ependymal cells also give rise to a smaller number of remyelinating oligodendrocytes after injury (Meletis et al., 2008). Injury-induced

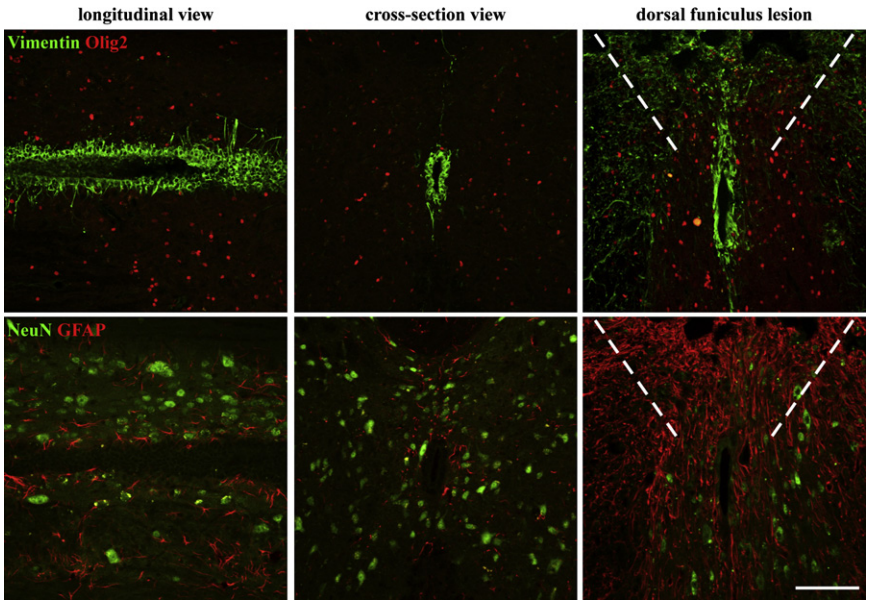

Figure 4. Endogenous Stem and Progenitor Cells in the Intact and Injured Spinal Cord
Cells in the ependymal layer, lining the central canal, have in vitro neural stem cell properties and generate mainly astrocytes in response to a spinal cord injury (Johansson et al., 1999; Meletis et al., 2008) (here an incision in the dorsal funiculus, lesion area marked by hatched lines). Parenchymal progenitors, many of which express Olig2, mainly generate oligodendrocytes after injury (Horky et al., 2006; Ohori et al., 2006). The left and middle panels are from uninjured animals, and the right panels are from an injured animal. Vimentin is expressed by ependymal cells and some reactive astrocytes; NeuN labels neurons; and GFAP, which is greatly induced after injury, labels astrocytes. Scale bar, 100 μm.

proliferation of ependymal cells is restricted to the lesioned segment, and a progressive ascending central canal dilatation rostral to the lesion with thinning of the ependyma and ependymal disruption has been described (Radojicic et al., 2007).

Proliferating cells in the parenchyma include NG2+/Olig2+/Nkx2.2+ progenitors (McTigue et al., 2001; Ohori et al., 2006; Yamamoto et al., 2001b) (Figure 3). Interestingly, BrdU labeling of cells proliferating in the uninjured spinal cord (i.e., mainly parenchymal progenitor cells) showed that this population is partly depleted by a lesion. The number of BrdU-labeled cells was greatly increased 1 month later compared to uninjured animals, suggesting some regeneration or long-lasting progeny of parenchymal progenitor cells (Horky et al., 2006). Retroviral labeling in the parenchyma showed that labeled cells, mainly NG2+ cells, give rise to progeny with more mature oligodendrocyte features (Horky et al., 2006; Ohori et al., 2006), and this is likely to be the main source of new oligodendrocytes.

Invading bone marrow-derived macrophages and granulocytes are also actively proliferating but represent a major fraction of the proliferating population only 1 week–1 month after injury (Horky et al., 2006; Kojima and Tator, 2000). Moreover, Schwann cells from the nerve roots can migrate into the lesion site and partially participate in the remyelination process (Talbott et al., 2005).

Can Endogenous Neural Stem and Progenitor Cells Be Modulated to Promote Recovery?

The main fate of the progeny of endogenous stem and progenitor cells after spinal cord injury is oligodendrocytes and astrocytes (Figure 4). The generation of oligodendrocytes by endogenous stem/progenitor cells contributes to remyelination and is likely to underlie some restoration of function (Keirstead and Blakemore, 1997). It is attractive to consider ways to facilitate this process. In contrast, the generation of astrocytes by endogenous stem/progenitor cells and the contribution to glial scar formation may potentially inhibit axonal regrowth. It is necessary to better understand the properties and functional role of cells derived from endogenous stem/progenitor cells in order to consider optimal strategies to modulate their response. Astrocytes are heterogeneous, and it is not presently clear to what degree different subtypes play different roles after injury. The glial scar is composed of both local astrocytes that become hypertrophic in response to the injury and new astrocytes derived from stem/progenitor cells that invade the lesion area. It is presently not clear whether these different astrocyte populations have different functions or affect the ability of regeneration differently.

In the same way as many transplantation strategies aim to promote remyelination of axons, the most readily apparent aim of modulating endogenous stem/progenitor cells may be to promote the differentiation of their progeny to oligodendrocytes and to increase the number of such cells. Several studies have indicated that it is possible to modulate the response of endogenous stem/progenitor cells. Intracerebroventricular infusion of FGF-2 alone or with EGF resulted in a marked increase in proliferation and nestin expression by ependymal cells (Kojima and Tator, 2002; Martens et al., 2002; Xu et al., 2006). Retroviral expression of Mash1 in parenchymal progenitor cells resulted in enhanced oligodendrocyte differentiation and maturation at the expense of astrocyte production, but these newly generated oligodendrocytes were no longer observed 1 month after injury (Ohori et al., 2006).

Transplantation studies have taught us that the fate of differentiating neural stem cell progeny is to a large degree dictated by the host environment, since the same cells may predominantly produce neurons if transplanted to a neurogenic niche but mainly astrocytes when grafted to a nonneurogenic region such as the spinal cord (Shihabuddin et al., 2000). Most studies have failed to detect neurogenesis in the adult rodent or primate spinal cord (Horky et al., 2006; Horner et al., 2000; Kojima and Tator, 2002; Mothe and Tator, 2005; Ohori et al., 2006; Yang et al., 2006). However, it appears that small numbers of neurons may be generated under certain circumstances (Danilov et al., 2006; Ohori et al., 2006). The strong influence of factors promoting glial differentiation in the adult spinal cord is apparent in neural stem cells engineered to ectopically express the proneural gene Neurogenin2; this results in close to complete neuronal differentiation in vitro but does not result in any large increase in the number of neurons when the cells are transplanted to the spinal cord (Hofstetter et al., 2005). Thus, the gliogenic cues in the adult spinal cord can override a strong intrinsic determinant of neuronal differentiation. The field of developmental neurobiology is rapidly increasing our knowledge of the molecular basis of fate choice, and this may aid in the development of strategies to promote oligodendroglial differentiation from endogenous stem/progenitor cells in the injured spinal cord.

Comparison of Different Potential Stem Cell-Based Repair Strategies

A pharmacological therapy modulating the response of endogenous neural stem cells to promote recovery would have an advantage over transplantation strategies in that it would be noninvasive. Transplantation of allogenic cells also calls for immunosuppression, with serious side effects. Immunoincompatibility could also result in rejection reactions, which may lead not only to the loss of the grafted cells, but also to inflammatory reactions that cause secondary damage. It remains unclear whether it may be possible to modulate neural stem cells in situ to accomplish a similar promotion of recovery, as seen after cell transplantation. Moreover, it is possible that the normal response to injury and scar formation is optimal in terms of regaining tissue integrity, preventing further damage, and containing inflammatory cells. Thus, modulating the response of endogenous neural stem cells to generate, for example, more remyelinating oligodendrocytes at the expense of scar-forming astrocytes may potentially pose a risk in decreasing the recovery of tissue integrity.

One uncertainty with the endogenous stem cell recruitment approach is the limited information from the adult human spinal cord. Stem cells isolated from the fetal human spinal cord recapitulate the integration, migration, and differentiation pattern of well-studied rodent stem cells upon transplantation into a rodent spinal cord injury (Yan et al., 2007). Furthermore, a recent study demonstrated the expansion of cells with in vitro neural stem cell properties from the adult human spinal cord (Dromard et al., 2008).

Different stem cell sources have potential advantages and disadvantages for transplantation. The main benefit of neural stem cells, compared to other stem cell sources for spinal cord repair,

lies in their commitment to produce neurons and glial cells. However, fetal neural stem cells require aborted human fetuses, which is a limited and ethically controversial source. Moreover, a therapy based on fetal tissue may be difficult to make widely available because of limited expansion capability, and that constitutes an impediment to making easily distributable cells. Adult neural stem cells are not as easily propagated to large numbers, and autologous therapies carry risks with cell harvesting and much work for each patient, making it impractical.

The majority of spinal cord injury patients suffer from chronic neuropathic pain, which decreases the quality of life (Siddall and Loeser, 2001). Transplantation of adult spinal cord neural stem cells promotes functional recovery but also increases neuropathic pain in rats (Hofstetter et al., 2005). The rats displayed signs of allodynia (a normally nonnoxious stimulus is perceived as painful), probably due to stem cell-derived astrocytes promoting sprouting of sensory axons. It is important in the evaluation of potential clinical trials to evaluate the risk of neuropathic pain. In the same way as these grafted cells may promote axonal sprouting to form new sensory circuitry, it is difficult to exclude the possibility that they also may promote the formation of other aberrant connections, which potentially could cause unwanted side effects.

The obvious benefit of nonneural somatic stem cells is their comparatively easy access for autologous use. However, if in vitro culture or selection is required, it immediately becomes more difficult to envisage a standardized large-scale therapy. Another caveat when considering bone marrow-derived cells for spinal cord repair is the limited mechanistic insight into how these cells may support recovery, which makes it difficult to predict the effect in the human situation.

ESC-derived myelinating glial progenitor cells can be derived with rather high efficiency and can likely be produced in close to unlimited quantities under standardized conditions. A caveat with using ESCs is that they are not autologous, requiring immunosuppression. A potential alternative to ESCs, avoiding the need for immunosuppression, may be patient-derived induced pluripotent stem cells (iPSCs) (Yamanaka, 2007). This would also circumvent the ethical concerns associated with ESCs. However, the required time for establishing iPSC lines may limit their use in the acute or subacute situation.

A major caveat to the use of ES-derived cells is the well-recognized risk of teratoma formation after transplantation. The risk of tumors is problematic in any stem cell therapy, but even more so when a very small tumor can cause large problems, as when growing in a limited space such as the spinal canal. Moreover, surgical removal of a tumor growing in the spinal cord, even though it is benign, is likely to result in considerable damage of bordering intact tissue. However, whereas it appears difficult to circumvent tumor formation after transplantation of ESCs differentiated toward a neuronal fate, teratomas have not yet been reported after transplantation of ESC-derived oligodendrocyte progenitors. ESC-derived cells currently appears to be the most attractive cell source for spinal cord repair, not least because of the possibility to scale up the production of cells and efficacy.

Perspectives

Spinal cord injury is often mentioned among the first possible new indications for future stem cell-based transplantation therapies (Vogel, 2005). In fact, a rather large number of spinal cord injury patients have already received transplants of stem cells and other cell types. In spite of the limited understanding of the efficacy, safety, and mechanisms by which bone marrow stem cells may promote spinal cord regeneration, phase I safety clinical transplantation trials have already been initiated (Callera and do Nascimento, 2006; Park et al., 2005; Tator, 2006; Yoon et al., 2007) and are today, without any study proving efficacy, offered commercially to patients at private clinics (see, for example, http://www.stemcellschina.com/ or http://www.xcell-center.com/). A group of independent scientists, not associated with any transplantation clinic, evaluated a small number of patients before and after treatment at a private clinic in China. They failed to find any positive effects of intraspinal injection of cultured cells from the brains of aborted human fetuses but found serious side effects in several of the patients, including meningitis in five out of seven patients (Dobkin et al., 2006). A thorough evaluation by an international consortium is being planned (Cyranoski, 2007).

The majority of spinal cord injury studies and initial evaluations of potential therapeutic strategies are typically performed in rodents. There are, however, important differences between the rodent and human spinal cord in, for example, the pathways of the motor systems and behavior. Although rodent studies are necessary and important, studies in nonhuman primates will in most cases be necessary to better predict both efficacy and safety in the human situation before entering clinical trials (Courtine et al., 2007). It will also be important to evaluate the outcome and potential side effects in experimental animals over much longer time courses than is often done, since patients in many cases are expected to live several decades after the treatment.

A stem cell-based transplantation strategy that proves successful in animal models and where mechanisms are understood still faces practical challenges to be translated to the clinic. It will need to be made easily accessible for hospitals, and this ideally includes the ability to bank cells and to be able to distribute them as an off-the-shelf product. The more simplified the cell handling techniques required by the clinic that will transplant the cells, the greater potential it has to become a widely used therapy.

Strategies to affect endogenous neural stem cells in the adult spinal cord today appear to be a more distant scenario. Efforts must be first put into acquiring a better knowledge of this stem cell reservoir. However, this is an exciting line of research that ultimately may result in pharmacological therapies circumventing the need for invasive and allogeneic strategies.

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